(3) Other alkaloids—Dissolve 0.25 g of Atropine Sulfate in 1 mL of diluted hydrochloric acid (1 in 10), add water to make 15 mL, and use this solution as the sample solution.

(i) To 5 mL of the sample solution add 2 to 3 drops of hydrogen hexachloroplatinate (IV) TS: no precipitate is formed.

(ii) To 5 mL of the sample solution add 2 mL of ammonia TS, and shake vigorously: the turbidity of the solution is not greater than that of the following control solution.

Control solution: To 0.30 mL of 0.01 mol/L hydrochloric acid VS add 6 mL of dilute nitric acid and water to make 50 mL. To this solution add 1 mL of silver nitrate TS, and allow 7 mL of the mixture to stand for 5 minutes.

(4) Hyoscyamine—Weigh accurately about 1 g of Atropine Sulfate, previously dried, and dissolve in water to make exactly 10 mL: the specific optical rotation $[\alpha]_D^{20}$ of this solution in a 100-mm cell is between $-0.60°$ and $+0.10°$.

(5) Readily carbonizable substances—Take 0.20 g of Atropine Sulfate, and perform the test: the solution has no more color than Matching Fluid A.

Loss on drying Not more than 4.0% (0.5 g, in vacuum, phosphorus (V) oxide, 110°C, 4 hours).

Residue on ignition Not more than 0.1% (0.5 g).

Assay Dissolve about 0.25 g of Atropine Sulfate, previously dried and accurately weighed, in 30 mL of acetic acid (100). If necessary, dissolve it by warming, and cool. Titrate with 0.05 mol/L perchloric acid VS until the color of the solution changes from purple through blue to blue-green (indicator: 3 drops of crystal violet TS). Perform a blank determination, and make any necessary correction.

Each mL of 0.05 mol/L perchloric acid VS
= 33.841 mg of $(C_{17}H_{23}NO_3)_2.H_2SO_4$

Containers and storage Containers—Tight containers.
Storage—Light-resistant.

Atropine Sulfate Injection

硫酸アトロピン注射液

Atropine Sulfate Injection is an aqueous solution for injection. It contains not less than 93% and not more than 107% of the labeled amount of atropine sulfate $[(C_{17}H_{23}NO_3)_2.H_2SO_4.H_2O$: 694.83].

Method of preparation Prepare as directed under Injections, with Atropine Sulfate.

Description Atropine Sulfate Injection is a clear, colorless liquid.

pH: 4.0 - 6.0

Identification **(1)** Evaporate a volume of Atropine Sulfate Injection, equivalent to 1 mg of Atropine Sulfate according to the labeled amount, on a water bath to dryness. Proceed with the residue as directed in the Identification (1) under Atropine Sulfate.

(2) Heat an exactly measured volume of Atropine Sulfate Injection, equivalent to 5 mg of Atropine Sulfate according to the labeled amount, on a water bath to a volume of 5 mL, and use this solution as the sample solution. Separately, weigh accurately 0.050 g of Atropine Sulfate Reference Standard, dissolve in ethanol (95) to make exactly 10 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under the Thin-layer Chromatography. Spot 50 μL of the sample solution and 10 μL of the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of chloroform and diethylamine (9:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly hydrogen hexachloroplatinate (IV)-potassium iodide TS on the plate: the spots obtained from the sample solution and the standard solution show a purple color and the same *Rf* value.

(3) Atropine Sulfate Injection responds to the Qualitative Tests for sulfate.

Assay To an exactly measured volume of Atropine Sulfate Injection, equivalent to about 5 mg of atropine sulfate $[(C_{17}H_{23}NO_3)_2.H_2SO_4.H_2O]$, add water to make exactly 100 mL, and use this solution as the sample solution. Separately, dissolve about 5 mg of Atropine Sulfate Reference Standard (determine previously its loss on drying in the same manner as directed under Atropine Sulfate), accurately weighed, in water to make exactly 100 mL, and use this solution as the standard solution. Pipet 4 mL each of the sample solution and standard solution, add 2 mL of sodium hydroxide TS, 10 mL of potassium hydrogen phthalate buffer solution, pH 5.6, and 20 mL of chloroform to each of the solutions, and shake vigorously for 5 minutes. Cool down to about 10°C, centrifuge, and collect the chloroform layer. Perform the test with these solutions as directed under the Ultraviolet-visible Spectrophotometry using chloroform as the blank. Determine the absorbances, A_T and A_S, of the subsequent solutions of the sample solution and the standard solution at 416 nm, respectively.

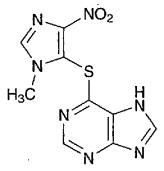
Amount (mg) of atropine sulfate $[(C_{17}H_{23}NO_3)_2.H_2SO_4.H_2O]$
= amount (mg) of Atropine Sulfate Reference Standard, calculated on the dried basis

$$\times \frac{A_T}{A_S} \times 1.0266$$

Containers and storage Containers—Hermetic containers.
Storage—Light-resistant.

Azathioprine

アザチオプリン



$C_9H_7N_7O_2S$: 277.26
6-(1-Methyl-4-nitro-1*H*-imidazol-5-ylthio)purine
[*446-86-6*]

Azathioprine, when dried, contains not less than 98.5% of $C_9H_7N_7O_2S$.

Description Azathioprine is light yellow crystals or crystalline powder. It is odorless.

It is sparingly soluble in *N,N*-dimethylformamide and in pyridine, very slightly soluble in water and in ethanol (99.5), and practically insoluble in chloroform and in diethyl ether.

It dissolves in sodium hydroxide TS and in ammonia TS.

It is gradually colored by light.

Melting point: about 240°C (with decomposition).

Identification **(1)** Dissolve 0.01 g of Azathioprine in 50 mL of water by warming. To 5 mL of this solution add 1 mL of dilute hydrochloric acid and 0.01 g of zinc powder, and allow to stand for 5 minutes: a yellow color is produced. Filter this solution: the filtrate responds to the Qualitative Tests for primary aromatic amines, and a red color is produced.

(2) Dissolve 0.01 g of Azathioprine in 50 mL of water by warming. To 1 mL of this solution add 0.5 mL of phosphotungstic acid TS and 0.5 mL of dilute hydrochloric acid: a white precipitate is formed.

(3) Prepare the test solution by proceeding with 0.03 g of Azathioprine according to the Oxygen Flask Combustion Method, using 20 mL of water as the absorbing liquid: the test solution responds to the Qualitative Tests (1) for sulfate.

(4) Dissolve 0.01 g of Azathioprine in 2 mol/L hydrochloric acid TS to make 100 mL. Dilute 5 mL of the solution with water to make 50 mL. Determine the absorption spectrum of the solution as directed under the Ultraviolet-visible Spectrophotometry, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Azathioprine Reference Standard prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

Purity **(1)** Clarity and color of solution—Dissolve 0.5 g of Azathioprine in 50 mL of *N,N*-dimethylformamide: the solution is clear and shows a light yellow color.

(2) Acid or alkali—Add 100 mL of water to 2.0 g of Azathioprine, shake well for 15 minutes, centrifuge for 5 minutes at 10,000 revolutions per minute, and filter. Discard the first 20 mL of the filtrate, add 2 drops of methyl red TS to 40 mL of the subsequent filtrate, and use this solution as the sample solution

(i) Add 0.10 mL of 0.02 mol/L hydrochloric acid VS to 20 mL of the sample solution: a red color develops.

(ii) Add 0.10 mL of 0.02 mol/L sodium hydroxide VS to 20 mL of the sample solution: a yellow color develops.

(3) Sulfate—To 25 mL of the filtrate obtained in (2) add 1 mL of dilute hydrochloric acid and water to make 50 mL, and perform the test using this solution as the test solution. Prepare the control solution with 0.40 mL of 0.005 mol/L sulfuric acid VS (not more than 0.038%).

(4) Heavy metals—Proceed with 2.0 g of Azathioprine according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(5) Arsenic—Prepare the test solution with 1.0 g of Azathioprine, according to Method 3, and perform the test using apparatus B (not more than 2 ppm).

(6) Related substances—Dissolve 0.010 g of Azathioprine in 80 mL of the mobile phase by warming, cool, add the mobile phase to make 100 mL, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add water to make exactly 100 mL, and use this solution as the standard solution. Perform the test with 20 μL each of the sample solution and the standard solution as directed under the Liquid Chromatography according to the following conditions. Determine each peak area of both solutions by the automatic integration method: the total area of the peaks other than that of azathioprine from the sample solution is not larger than 1/2 of the peak area of azathioprine from the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 296 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: Adjust to pH 2.5 of a solution of 0.05 mol/L potassium dihydrogenphosphate TS (1 in 2) with diluted phosphoric acid (3 in 2000). To 800 mL of this solution add 200 mL of methanol.

Flow rate: Adjust the flow rate so that the retention time of azathioprine is about 8 minutes.

Time span of measurement: About three times as long as the retention time of azathioprine after the solvent peak.

System suitability—

Test for required detection: To exactly 5 mL of the standard solution add water to make exactly 50 mL. Confirm that the peak area of azathioprine obtained from 20 μL of this solution is equivalent to 8 to 12% of that of azathioprine obtained from 20 μL of the standard solution.

System performance: Dissolve 0.010 g of Azathioprine in 80 mL of water by warming, cool, and add water to make 100 mL. To 2 mL of this solution add 2 mL of a solution, separately prepared by dissolving 0.06 g of benzoic acid in 3 mL of methanol and diluting with water to make 10 mL, and add the mobile phase to make 25 mL. When the procedure is run with 20 μL of this solution under the above operating conditions, azathioprine and benzoic acid are eluted in this order with the resolution between these peaks being not less than 9.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak areas of azathioprine is not more than 2.0%.

Loss on drying Not more than 0.5% (1 g, 105°C, 5 hours).

Residue on ignition Not more than 0.10% (1 g).

Assay Weigh accurately about 0.5 g of Azathioprine, previously dried, add 80 mL of *N,N*-dimethylformamide, and warm to dissolve. After cooling, titrate with 0.1 mol/L tetramethylammonium hydroxide VS until the color of the solution changes from yellow through yellow-green to blue-green (indicator: 1 mL of thymol blue-dimethylformamide TS). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L tetramethylammonium hydroxide VS
= 27.727 mg of $C_9H_7O_7O_2S$

Containers and storage Containers—Well-closed containers.

Storage—Light-resistant.

Azathioprine Tablets

アザチオプリン錠

Azathioprine Tablets contain not less than 95% and not more than 105% of the labeled amount of azathioprine ($C_9H_7N_7O_2S$: 277.26).

Method of preparation Prepare as directed under Tablets, with Azathioprine.

Identification **(1)** Weigh a quantity of powdered Azathioprine Tablets, equivalent to 0.01 g of Azathioprine according to the labeled amount. Add 50 mL of water, shake well while warming, and filter. Proceed with 5 mL of the filtrate as directed in the Identification (1) under Azathioprine.

(2) Proceed with 1 mL of the filtrate obtained in (1) as directed in the Identification (2) under Azathioprine.

(3) Determine the absorption spectrum of the sample solution in the Assay as directed under the Ultraviolet-visible Spectrophotometry: it exhibits a maximum between 278 nm and 282 nm.

(4) Weigh a quantity of powdered Azathioprine Tablets, equivalent to 0.1 g of Azathioprine to the labeled amount. Add 10 mL of a solution of ammonia solution (28) in methanol (1 in 10), shake well, filter, and use the filtrate as the sample solution. Separately, dissolve 0.1 g of Azathioprine Reference Standard in 10 mL of a solution of ammonia solution (28) in methanol (1 in 10), and use this solution as the standard solution. Perform the test with these solutions as directed under the Thin-layer Chromatography. Spot 5 μL each of the sample solution and the standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of chloroform, a solution of ammonia solution (28) in methanol (1 in 10), *n*-butyl formate and 1,2-dichloroethane (15:10:5:2) to a distance of about 15 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots from the sample solution and the standard solution show the same *Rf* value.

Assay Weigh accurately and powder not less than 20 Azathioprine Tablets. Weigh accurately a portion of the powder, equivalent to about 0.1 g of azathioprine ($C_9H_7N_7O_2S$), add 20 mL of dimethylsulfoxide for ultraviolet-visible spectrophotometry, shake well, add 0.1 mol/L hydrochloric acid TS to make exactly 500 mL, and filter. Discard the first 20 mL of the filtrate, measure exactly 3 mL of the subsequent filtrate, add 0.1 mol/L hydrochloric acid TS to make exactly 100 mL, and use this solution as the sample solution. Separately, weigh accurately about 0.1 g of Azathioprine Reference Standard, previously dried at 105° C for 5 hours, dissolve in 20 mL of dimethylsulfoxide for ultraviolet-visible spectrophotometry, and add 0.1 mol/L hydrochloric acid TS to make exactly 500 mL. Measure exactly 3 mL of this solution, add 0.1 mol/L hydrochloric acid TS to make exactly 100 mL, and use this solution as the standard solution. Determine the absorbances, A_T and A_S, of the sample solution and the standard solution at 280 nm as directed under the Ultraviolet-visible Spectrophotometry, respectively.

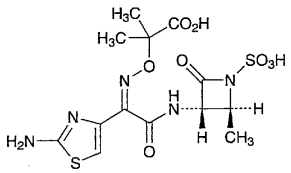
Amount (mg) of azathioprine ($C_9H_7N_7O_2S$)
= amount (mg) of Azathioprine Reference Standard
$\times \frac{A_T}{A_S}$

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Aztreonam

アズトレオナム



$C_{13}H_{17}N_5O_8S_2$: 435.43
2-{(*Z*)-(2-Aminothiazol-4-yl)-[(2*S*,3*S*)-2-methyl-4-oxo-1-sulfoazetidin-3-ylcarbamoyl]-methyleneaminooxy}-2-methyl-1-propanoic acid
[*78110-38-0*]

Aztreonam contains not less than 920 μg (potency) per mg, calculated on the anhydrous basis. The potency of Aztreonam is expressed as mass (potency) of aztreonam ($C_{13}H_{17}N_5O_8S_2$).

Description Aztreonam occurs as a white to yellowish white crystalline powder.

It is freely soluble in dimethylsulfoxide, slightly soluble in water and in methanol, and very slightly soluble in ethanol (95).

Identification **(1)** Determine the absorption spectrum of a solution of Aztreonam (3 in 100,000) as directed under the Ultraviolet-visible Spectrophotometry, and compare the spectrum with the Reference Spectrum or the spectrum of Aztreonam Reference Standard: both spectra exhibit similar intensities of absorption at the same wavelength.

(2) Determine the spectrum of a solution of Aztreonam in deuterated dimethylsulfoxide for nuclear magnetic resonance spectroscopy (1 in 10), using a light hydrogen substance existing in deuterated dimethylsulfoxide for nuclear magnetic resonance spectroscopy as an internal reference compound and 2.50 ppm for its chemical shift, as directed under the Nuclear Magnetic Resonance Spectroscopy (^1H): it exhibits a multiple signal at around δ 1.5 ppm, and a single signal at around δ 7.0 ppm. The ratio of integrated intensity of each signal is 9:1.

Optical rotation $[\alpha]_D^{20}$: −26 – −32° (0.25 g calculated on the anhydrous bases, water, 50 mL, 100 mm).

pH Dissolve 0.05 g of Aztreonam in 10 mL of water: the pH of this solution is between 2.2 and 2.8.